Lebensm.-Wiss. u.-Technol., 25, 365–370 (1992)





# Hydration Effects on the Thermal Stability of Proteins in Cracked Soybeans and Defatted Soy Flour

**David J. Sessa**

Biopolymer Research Unit, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Agricultural Research Service, Peoria, IL 61604 (U.S.A.)

*(Received April 8, 1992; accepted May 1, 1992)*

*Hydration effects on the thermal stability of soybean 7S (β-conglycinin) and 11S (glycinin) storage proteins in cracked, dehulled soybeans and dehulled, defatted soy flour were examined by differential scanning calorimetry. In model systems with pure 7S and 11S and Kunitz trypsin inhibitor (KTI), the denaturation temperatures ($T_d$) increased with decrease in moisture, from about 70% to dry state according to their respective regression curves: $82.0 + 124.1e^{-0.075M}$, $95.0 + 138.2e^{-0.087M}$, and $76.3 + 125.4e^{-0.081M}$, where M equals percent moisture (wet basis). In cracked soybeans and defatted soy flour with endogenous moistures of 9.09 and 9.50%, respectively, endotherms for 7S protein were observed at 132.8 and 155.6 °C and for 11S protein at 160.3 and 168.9 °C. Calculated $T_d$s at 9.1% moisture were 144.8 and 157.6 °C, respectively. Differences in $T_d$s of experimental and calculated values over the range of moistures from about 9 to 60% were attributed to the microenvironment surrounding the proteins, which may create non-equilibrium moisture conditions around the proteins. Though an endotherm for KTI was not observed at the endogenous bean moisture, it became evident at intermediate moistures of 20 to 33% where this endotherm was distinct from the other two storage proteins. The enthalpy of reaction (ΔH) for 7S, 11S and KTI did not change in the model systems over the moisture range from dry state to 70%. However, a decrease in 11S ΔH was observed in the cracked bean and defatted soy flour systems when moisture increased above 9%. With 7S, we observed peak spreading and diminished ΔH at the endogenous moisture of cracked beans and upon further drying of the defatted soy flour. Above 28% moisture we again observed a decrease in 7S ΔH. These decreases in ΔH for 7S and 11S may be attributed to the effect of non-protein or protein components interacting with the storage proteins.*

## Introduction

Thermal denaturation of soybean proteins in solution has been extensively studied. Yet, very little is known about the heat stability and denaturation of proteins under low to intermediate moisture conditions. This information is basic to food processing and manufacturing, particularly in extrusion cooking where foodstuffs, including protein mixtures, are subjected to a variety of heat treatments under low to intermediate moisture conditions. Knowledge of the thermal behavior of plant proteins during extrusion, retorting, toasting and frying permits process design to achieve products with different flavor and textural attributes.

Differential scanning calorimetry (DSC) has been used extensively as a tool in food research to study the thermodynamics and kinetic properties of protein denaturation in solution and solid states. Arntfield and co-workers (1,2) applied this technology to investigate the effects of moisture on the thermal stability of fababean and soybean storage proteins. Sheard and co-workers (3) investigated the effects of moisture on the thermal denaturation of 7S and 11S proteins in soybean protein isolates. Oates *et al.* (4) and Oates and Ledward (5) studied the effects of hydrocolloids on soybean storage proteins under low moisture conditions. Kitabatake *et al.* (6) examined the denaturation of purified soybean 7S and 11S proteins under low moisture conditions. Vooijs *et al.* (7) analysed the effects of water content and processing time on the inactivation of pure soybean Kunitz trypsin inhibitor (KTI).

In the literature cited above, the relationship between the thermal denaturation parameters in purified soy proteins and protein mixtures as they exist in the cracked bean and defatted flour has not been determined. The objective of our work was to investigate the effects of low to intermediate moistures on the thermal stability of soy storage proteins as they exist in cracked full-fat soybeans and defatted soy flour. Model systems, containing purified soybean 7S and 11S storage proteins and KTI, were used as a reference to determine the effects of the microenvironment surrounding these three proteins in cracked beans and defatted flour.

## Materials and Methods

### *Materials*

Whole soybeans, Century variety, were cracked into 4 to 6 parts by passage through cracking rolls, and dehulled with a box aspirator. The cracked beans were flaked, then hexane-defatted, air-dried and ground to yield a flour with a nitrogen solubility index of 73. Dr Walter Wolf, NCAUR, kindly provided freeze-dried, electrophoretically pure soybean 7S and 11S storage proteins prepared from Raiden variety soybeans (8). Soy KTI, type 1-S, was purchased from Sigma Chemical Co., St Louis, MO. All other chemicals were reagent grade.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

0023-6438/92/040365 + 06 $08.00/0 

*Treatments*

Cracked beans were rinsed with distilled water at a bean to solvent ratio of 1:5 to increase moisture level in the beans. To achieve higher percent moistures, the cracked beans were steeped either at room temperature (30 °C) or heated at 65 °C in Erlenmeyer flasks for up to 2 h at a 1:5 bean to solvent ratio. The steeped, cracked beans were drained, then placed on paper towels for 30 min prior to bottling in a sealed container and refrigerated at 4 °C until used. Defatted soy flour (5 to 10 mg) was weighed into tared DSC aluminum pans and covers and heated uncovered in the DSC apparatus under dry nitrogen at 110 °C for 1 h to pasteurize the sample. Moistures of the weighed samples were adjusted by micropipetting weighed amounts of distilled water onto the inner portion of the DSC aluminum pan cover prior to sealing and reweighing. Each sample was equilibrated at room temperature for 14 to 16 days prior to analyses. Percent moisture in the sample at time of analysis was then calculated on a wet solids basis. Moistures of the purified proteins were adjusted similarly to the defatted soy flour samples. However, with these sealed cells equilibration times were 18 to 64 h at room temperature. For all samples, the moisture content of the original material was taken into account in calculating the moisture content of each system.

Moisture content of cracked beans and defatted flour samples was measured as per cent moisture (g/kg) by weight difference before and after drying under vacuum for 18 h at 80 °C. To dry the pure protein samples, an open aluminum DSC pan containing a weighed amount of protein was heated in the DSC to 60 °C for KTI, 115 °C for 7S and 125 °C for 11S and held at this temperature in a dry nitrogen atmosphere for various time intervals, from 5 min to 2.5 h prior to cooling, sealing and reweighing. When the moisture loss was constant for a given weight of sample, the dried weight was considered as 'apparent' zero moisture. With all the pure protein denaturation runs, the pans were punctured and reheated to the above temperatures until weight was constant in order to record moisture loss of the denatured proteins.

*Analyses*

Thermal denaturation of soy samples with various moisture levels was studied with a Perkin-Elmer DSC 7 using a TAS 7 software package. Aluminum pans were packed with 7 to 10 mg cracked soy beans that were sliced into thin segments with a razor blade or with 2 to 5 mg purified protein prior to sealing with a crimped aluminum cover. Samples and an empty crimped aluminum reference pan were scanned at a rate of 5.0 °C/min from 50 to 250 °C. Temperature calibration and enthalpy of denaturation ($\Delta H$) for the DSC cell were determined with a weighed sample of indium at the above scan rate and temperature range. Thermal denaturation of the protein gave an endotherm and $T_d$ was used to signify the peak maximum. The $\Delta H$ was calculated in joules per unit weight of dry protein. Total protein in cracked soybeans and defatted soy flour was determined from Kjeldahl nitrogen values (N × 6.25).

## Results and Discussion

*Thermal denaturation of pure soy proteins*

The moisture content of freeze-dried, purified soy proteins was determined by heating each protein in an open aluminum DSC pan under dry nitrogen to a specified temperature and holding at this temperature over time intervals until moisture loss was constant. Freeze-dried KTI readily lost its moisture by holding at 60 °C for 30 min, to give a constant per cent moisture loss of 8.4 ± 0.1 (mean ± standard deviation); freeze-dried 7S protein reached constant moisture loss after only 5 min heating at 115 °C and its percent moisture loss was 11.0 ± 0.2; whereas, 11S protein reached its constant moisture loss after 2.5 h heating at 125 °C, which resulted in a percent moisture loss of 11.5 ± 0.1. KTI and 7S protein not only readily lost moisture with heating but also readily rehydrated when moisturized and equilibrated for 18 h at room temperature. In contrast, the 11S required excessive heating and time to lose its moisture and when remoisturized above 20% required at least 64 h equilibration at room temperature. Otherwise, if not equilibrated, 11S gave a double endotherm. After each DSC denaturation run the aluminum pan lids were punctured and then reheated in the DSC unit, held at the specified temperatures until weight loss was constant. The denatured proteins lost additional per cent moisture: 6.5 for KTI, 3.7 for 7S and 3.0 for 11S. The conformational changes that occurred during denaturation allowed a further loss of moisture.

Thermal curves for proteins at 'apparent' zero moisture are shown in **Fig. 1**. Endotherms were observed at $T_d$s 203.6 ± 0.5 °C for KTI, 218.5 ± 0.6 °C for 7S protein and 210.2 ± 0.2 for 11S protein. The endotherm for 7S was over 8 °C higher than that of the 11S protein. The higher heat stability of 7S when compared to 11S at zero moisture is unusual and may reflect the carbohydrate content of 7S conferring greater stability, since 11S protein has no carbohydrate. Kitabatake *et al.* (6) observed a disappearance of the soy 7S protein endotherm at low moisture which they attributed to peak spreading as the water content decreased. We did not observe this behavior with our purified 7S. However, we did observe peak spreading and diminished $\Delta H$ in our defatted soy flour system. Apparently, the 7S of Kitabatake *et al.* (6) contained some impurity that caused peak spreading at diminished moisture content. All three soy proteins showed high thermal stability in the dried state (**Fig. 1**) which indicates that purified soy proteins in this state can resist denaturation when processed at temperatures up to 190 °C.

The three curves in **Fig. 2**, which demonstrate the effect of

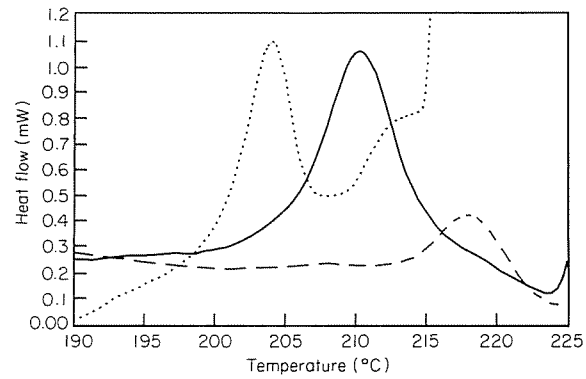


**Fig. 1** Differential scanning calorimetric thermograms of dehydrated, pure soy proteins. (---), 7S; (——), 11S; (------), KTI

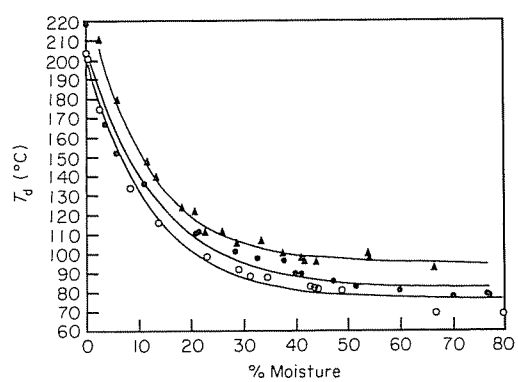


**Fig. 2** Effect of moisture on thermal denaturation of pure soy proteins: ▲, 11S (glycinin); ●, 7S (β-conglycinin); ○, KTI

moisture (recorded as percent moisture in the undenatured proteins) on the thermal denaturation of pure soy proteins, were best-fit by iterative (Gaussian) methods and derived as an asymptote function according to the NLIN procedure in SAS (9) obtained as follows:

$$\frac{dT}{dM} = k(T - A)$$

$$\frac{dT}{T - A} = k dM$$

$$\ln(T - A) = \ln B - kM$$

$$T - A = Be^{-kM}$$

$$T = A + Be^{-kM}$$

where $T = T_d$ denaturation temperature endotherm in °C, $A$ = asymptote of denaturation temperature as moisture increased, $B$ = the denaturation temperature which in combination with $A$ gives the $T_d$ in °C at constant moisture loss recorded as near 0% for the undenatured protein, $M$ = percent moisture and $k$ = constant by which each change in moisture affects $T_d$.

Equations estimated were:

KTI: $T_d$ (°C) = $76.3 + 125.4e^{-0.081}M$
7S protein: $T_d$ (°C) = $82.0 \times 124.1e^{-0.075}M$
11S protein: $T_c$ (°C) = $95.0 + 138.2e^{-0.087}M$

Thus, 11S protein had the greatest intercept ($A + B = 233.2$) and a $k$ value of −0.087. KTI and 7S had similar intercepts of 201.6 and 206.1, respectively, and $k$ values of −0.081 and −0.075. Overall, the theoretical value of $T_d$ was in the range of 200 to 235 °C at moisture per cent near zero and between 75 to 95 °C at high values of moisture. The rate of decrease in $T_d$ at any point, as moisture was increased by 1%, was near 7.5 to 9% of the difference between $T_d$ in °C at that point and the minimum $T_d$ in °C. The standard error for the derived values is given in **Table 1**.

In **Fig. 2** the $T_d$s of the pure proteins were not affected by water content in the 30 to 70% range. Sheard *et al.* (3) found a similar transition point in their studies with soy flour and soy protein isolate. Likewise, Vooijs *et al.* (7) observed a similar transition point with soy KTI. Tanteeratarm *et al.* (10) observed a biphasic linear vapor sorption isotherm for both 7S and 11S soy proteins at 0.28 and 0.26 g water/g solid. These authors could distinguish the two states of bound water, namely polymer and capillary water, via pulsed nuclear magnetic resonance (NMR). Apparently, we observed a similar, but not as distinct, break via our DSC analyses. All of our observed $T_d$s for 7S, 11S and KTI were higher than those observed by either Sheard *et al.* (3) or Vooijs *et al.* (7). For example, at 10 to 13% moisture our respective values for 7S, 11S and KTI were approximately 26, 11 and 27 °C higher than the literature values (3,7). However, at 11% moisture our 11S protein endotherm was about 32 °C lower than that recorded by Kitabatake *et al.* (6). Some of these discrepancies in $T_d$s can be related to the method used for moisture determination, e.g. heating for a constant weight versus Karl Fischer moisture analysis and whether the moisture was based on dry or wet sample weight. We did observe a further loss of moisture in all three proteins upon denaturation which we did not account for in **Fig. 2**. Method of isolation, protein purity and presence of trace amounts of salts or mercaptoethanol used in the isolation may also affect the $T_d$s.

**Table 1** Standard error for values in derived equations for **Fig. 2**

| Protein | $A$ | SE | $B$ | SE | $k$ | SE |
|---|---|---|---|---|---|---|
| KTI | 76.3 | 2.1 | 125.4 | 3.3 | −0.081 | 0.006 |
| 7S | 82.0 | 2.7 | 124.1 | 5.2 | −0.075 | 0.008 |
| 11S | 95.0 | 1.6 | 138.2 | 4.5 | −0.087 | 0.006 |

**Table 2** Effect of moisture on the enthalpy of denaturation (ΔH) of soy Kunitz trypsin inhibitor (KTI), 7S and 11S storage proteins

| Sample process[a] | ΔH J/g[b] | | |
|---|---|---|---|
| | KTI | 7S | 11S |
| A | 10.7 ± 1.7 (2) | 11.2 ± 0.5 (4) | 26.3 ± 1.3 (5) |
| B | 12.5 ± 2.0 (14) | 11.7 ± 0.6 (16) | 11.7 ± 1.1 (15) |

[a] A = Freeze-dried, then thermally dried to constant moisture; B = freeze-dried, then water added up to 77% moisture content.
[b] Each value is the mean ± standard deviation of ($n$) replicates.

The effect of moisture on the ΔH of the soy proteins remained constant over the 0 to 70% moisture range except for the 11S protein (**Table 2**). The decrease in ΔH from 26 to 12 needs further investigation to explain this change. However, a possible explanation may be that we are observing the 11S protein at different states of oxidation and polymerization. Freeze-drying the 11S protein will cause a decrease in surface and internal SH bonds along with a respective increase in SS bonds (11). Storage of freeze-dried 11S protein at relative humidities of 11 and 96% caused protein polymerization, mainly through disulfide bond participation (12,13). The more highly polymerized 11S protein may thus require less thermal energy, hence lower ΔH, for its denaturation. The thermal drying of freeze-dried 11S protein at 125 °C may have caused increase in free SH groups and a decrease in half-cystine content similar to that observed for heated soy protein isolate gels (14). Marshall and Zarins (15) reported a ΔH for 11S protein at 1.0% concentration in the presence of 2-mercaptoethanol of 24.0 J/g, while, at the same protein concentration, but in the absence of 2-mercaptoethanol, ΔH was 7.4 J/g. Remoisturizing our thermally dried 11S at different percentages gave ΔHs equal to that of the thermally dried samples. Therefore, when we thermally dried or thermally dried and remoisturized our 11S, we may have changes its oxidation state to a more reduced form. Our value for pure 11S, when thermally dried, or thermally dried and remoisturized, of 26.3 J/g is similar to those values reported in the literature (6,16). The ΔH of our 7S protein was over twice that of the literature value (6) and may reflect its high purity and/or method of isolation. The lack of change in ΔH with moisture content from 0 to 70% for all the soy proteins confirmed the findings of Kitabatake *et al.* (6) for 7S and 11S proteins and is unusual since ΔH generally decreases with decreasing water content (17–19). However, tropocollagen, exhibited an increase in ΔH as the water content decreased from 60 to 28% wet sample basis, although a further reduction in water content caused a rapid decrease (20). Further research is needed to explain the lack of change in ΔH for pure soy proteins under varying degrees of hydration.

*Thermal denaturation of proteins in cracked soybeans and defatted soy flour*

Cracked soybeans were readily hydrated from 9.1 to 25.4% simply by rinsing the beans with water at 65 °C immediately followed by draining. To obtain per cent moistures below 25%, room temperature rinses or short steep times up to 5 min were required. Steeping at 65 °C for 15 min hydrated our beans to 56.1% which increased to 66.0% within 2 h (**Fig. 3**). Thermal curves of cracked beans with moisture contents of 25.4, 33.0 and 66.0% are shown in **Fig. 4**. At 25.4 and 33.0% moisture, the endotherms for KTI were evident at 92.1 and 79.9 °C, respectively, while at 66.0 and 9.1% (not shown) moistures no endotherms were evident. KTI is a minor protein in soy and can readily be lost as part of the baseline at the

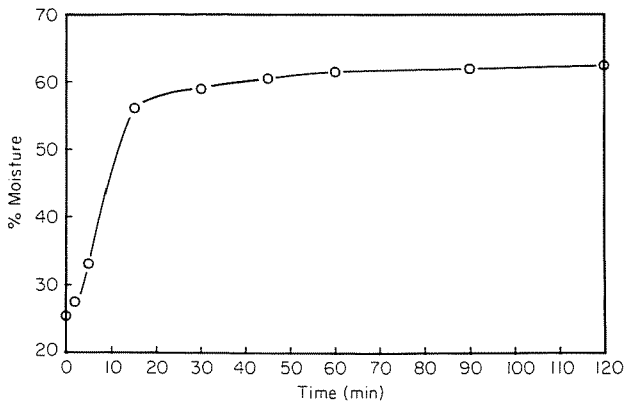


Fig. 3 Effect of steep time at 65 °C on water uptake of cracked soybeans

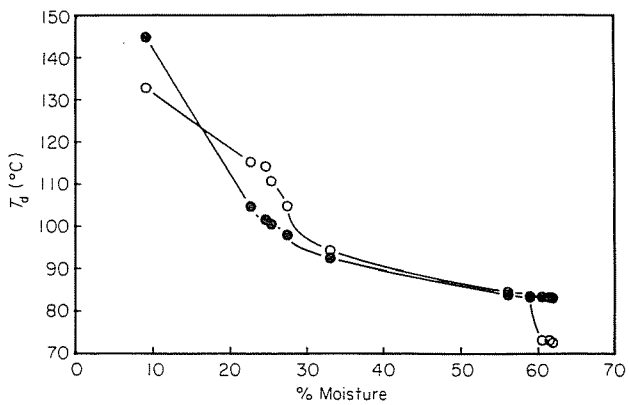


Fig. 5 Effect of moisture on the thermal denaturation of 7S storage protein in cracked soybeans: ○, experimental; ●, calculated

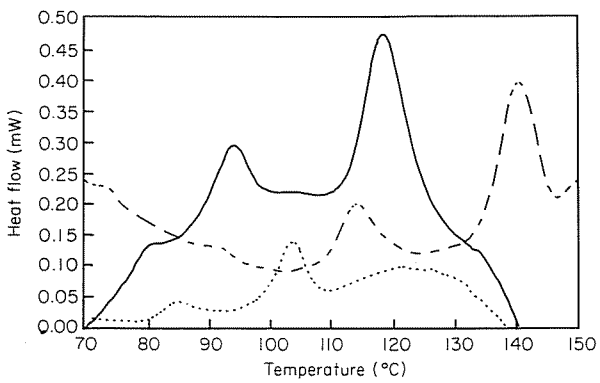


Fig. 4 Differential scanning calorimetric thermograms of moisturized cracked soybeans. Moisture (%): (---), 25.4; (——), 33.0; (-----), 66.0

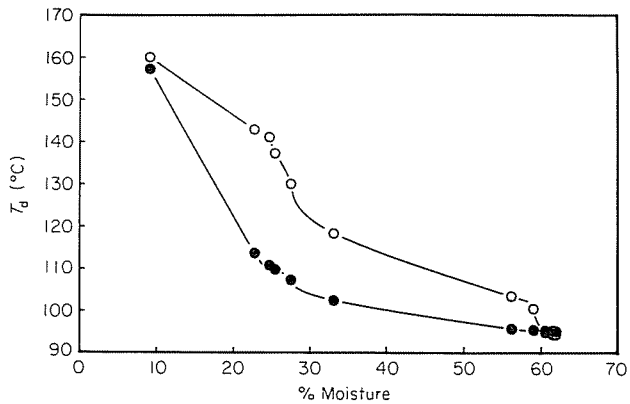


Fig. 6 Effect of moisture on the thermal denaturation of 11S storage protein in cracked soybeans: ○, experimental; ●, calculated

sensitivities used in this study or is coincidental with 7S protein endotherm. If we use our regression curve for KTI in the model system, an endotherm for KTI in the cracked bean system was 136.4 °C at 9.1% moisture, 92.4 °C at 25.4%, 85.0 °C at 33.0% and 76.9 °C at 66.0% moisture. Model system endotherms for KTI at 25.4 and 33.0% moistures are close but not coincidental with our experimental results and most likely reflect the impact of other components in the cracked beans or flour on $T_d$.

Experimental values for denaturation endotherms of 7S and 11S in cracked beans differed from those of the purified proteins (**Figs 5** and **6**). Water equilibration may be the limiting factor when $T_d$ in cracked beans is compared to $T_d$s of purified proteins. The $T_d$s for soy 7S and 11S, for the most part, parallel each other over per cent moistures from 9 to 60%. In general, the experimental $T_d$s are higher than the calculated values. Apparently, we are observing a competition for $H_2O$ between 7S, 11S and non-protein constituents in the cracked soybeans, where limited water reaching the protein would cause higher denaturation temperatures. Sheard *et al.* (3) observed that a soy flour system gave higher peak denaturation temperatures than the isolates prepared from these flours, particularly when moistures were below 60%. They attributed the differences in $T_d$s of flour versus isolate to carbohydrate–water and carbohydrate–protein interactions. The carbohydrates would more successfully compete for the available moisture and result in higher heat stability for the proteins. As discussed with purified proteins, the 7S protein more readily hydrates and dehydrates than the 11S protein. If we assume that the 7S hydrates more readily than the 11S in cracked beans, the $T_d$s for 7S in cracked beans and purified 7S should be closer than the $T_d$s for 11S in cracked beans and purified 11S. This becomes obvious when we compare data points from **Figs 5** and **6**. At the initial moisture content of our seeds (9.1%), $T_d$ for 7S was 132.8 °C versus a calculated value for purified 7S of 144.8 °C, whereas 11S was 160.3 °C in the seed versus 157.6 °C for purified protein. The 12.0 °C downward shift in experimental $T_d$ for 7S will be better demonstrated in our investigation of defatted soy flour systems with more data points at the lower moistures.

As can be seen in **Figs 7** and **8**, the $T_d$s for the storage proteins in the defatted soy flour are similar to the $T_d$s of 7S and 11S in the cracked beans shown in **Figs 5** and **6**. At about 25% moisture there exists about a 15 °C difference between purified 7S and 7S in soy flour and about a 30 °C difference in $T_d$s for 11S (**Figs 7** and **8**). At about 9% moisture, the $T_d$s for 7S and 11S in defatted soy flour are similar to their respective $T_d$s for the purified proteins. Below 9%, these same proteins

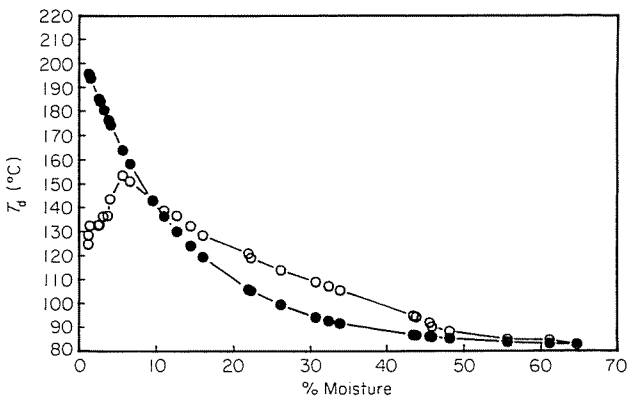


Fig. 7 Effect of moisture on the thermal denaturation of 7S storage protein in defatted soy flour: ○, experimental; ●, calculated

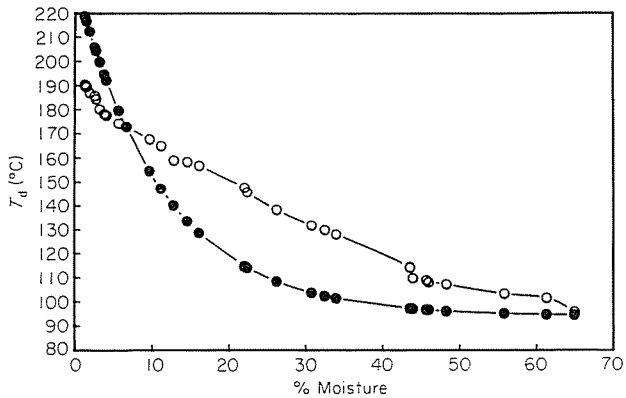


**Fig. 8** Effect of moisture on the thermal denaturation of 11S storage protein in defatted soy flour: ○, experimental; ●, calculated

in flour destabilize with each showing a drop in thermal denaturation temperature. The endotherm for 7S in the flour showed increased peak broadening and finally disappearance of the peak. Kitabatake *et al.* (6) likewise observed disappearance of endotherms for their semi-purified soy 7S. Their observed disappearance occurred at about 11% moisture. The cause of this 7S peak broadening needs further investigation. Since the $T_d$ for 11S in flour also shows marked change from the $T_d$ in purified 11S at similar moisture content, an interaction of the two storage proteins may be occurring at low moisture conditions.

With purified proteins, we did not observe any change in $\Delta H$ over the 0 to 70% moisture range. As can be seen from the values in **Table 3** for cracked soybeans and defatted soy flour, the $\Delta H$s for 7S protein increased from a low of 1.0 J/g in the 1 to 3% moisture range for defatted soy to a high of 4.7 ± 0.1 J/g protein at 11 to 28% moisture range for cracked beans, and then decreased as moistures increased to 65%. In contrast, the $\Delta H$ for 11S proteins in both cracked soybean and defatted soy flour consistently dropped when moistures increased to 65%. Baselines for thermal curves of 11S protein at moisture levels below 10% were poorly defined and could only be estimated. Oates *et al.* (4) likewise observed an increase in $\Delta H$ for 7S protein with increasing water content to about 31% water, with a subsequent decrease as the water content increased further. From their data, we estimated $\Delta H$ at 10% moisture as 3.1 J/g protein, which increased to 3.6 J/g protein at 31% moisture and then decreased to 1.3 J/g protein at about 60% moisture. These values are consistent with our findings. These authors did not estimate the $\Delta H$s of 11S protein because the baseline on the thermogram was difficult to determine following its denaturation.

According to Tanteeratarm *et al.* (10), there is a change in state of water for 7S at 0.28 g water/g solid. Apparently, at moisture contents above this level the 7S requires less heat input to effect its denaturation. Upon reducing the moisture of 7S samples, we observed a significant drop in $\Delta H$ for the defatted soy flour system. This drop is consistent with others

**Table 3** Effect of moisture on the denaturation enthalpy ($\Delta H$) of cracked soybean and soy flour storage proteins

| Moisture range (%) | 7S, $\Delta H$ (J/g protein)[a] | | 11S, $\Delta H$ (J/g protein)[a] | |
|---|---|---|---|---|
| | Cracked bean | Soy flour | Cracked bean | Soy flour |
| 1–3 | — | 1.0 ± 0.2 | — | 21.9 ± 1.4 |
| 4–10 | 4.0 ± 0.2 | 3.5 ± 0.4 | 10.3 ± 0.4 | 19.7 ± 1.4 |
| 11–28 | 4.7 ± 0.1 | 3.9 ± 0.4 | 9.7 ± 0.3 | 13.4 ± 0.3 |
| 29–34 | 2.8 ± 0.1 | 2.9 ± 0.2 | 8.6 ± 0.4 | 12.0 ± 0.3 |
| 35–65 | 1.5 ± 0.1 | 0.6 ± 0.2 | 4.8 ± 0.3 | 5.6 ± 1.2 |

[a] Each value is the mean ± standard deviation.

(12–15) who have studied the effects of hydration on purified proteins, but is inconsistent with our findings for purified soy 7S. The drop in $\Delta H$s of 11S in cracked soybeans and soy flour is likewise inconsistent with our findings for purified 11S. Both protein and non-protein constituents may play a major role in defining $T_d$s in cracked soybeans and soy flour at a variety of moisture contents.

Our research not only confirms some of the trends and reported literature values for $T_d$s and $\Delta H$s of soy proteins and points out differences where they exist, but also now allows a reasonable degree of predictability for the thermal denaturatiton of soy proteins in more complex systems when subjected to various degrees of hydration. We also demonstrated that $\Delta H$ differences in purified 7S and 11S and cracked beans/soy flour may now be used to assess the effects of other components on these proteins. To further investigate these effects our future work will involve the evaluation of neutral and reducing salts and carbohydrates on thermal denaturation of soybean proteins at low and intermediate moisture levels.

## References


1 Arntfield, S. D. and Murray, E. D. The influence of processing parameters on food protein functionality. I. Differential scanning calorimetry as an indicator of protein denaturation. *Canadian Institute of Food Science and Technology Journal*, **14**, 289–294 (1981)

2 Arntfield, S. D., Murray, E. D. and Ismond, M. A. H. The influence of processing parameters on food protein functionality. III. Effect of moisture content on the thermal stability of fababean protein. *Canadian Institute of Food Science and Technology Journal*, **18**, 226–232 (1985)

3 Sheard, P. R., Fellows, A., Ledward, D. A. and Mitchell, J. R. Macromolecular changes associated with the heat treatment of soya isolate. *Journal of Food Technology*, **21**, 55–60 (1986)

4 Oates, C. G., Ledward, D. A. and Mitchell, J. R. Physical and chemical changes resulting from heat treatment of soya and soya alginate mixtures. *Carbohydrate Polymers*, **7**, 17–33 (1987)

5 Oates, C. G. and Ledward, D. A. Thermal behavior of soya 7S globulin: effect of moisture content and added hydrocolloid on the denaturational change in heat capacity. *Food Chemistry*, **40**, 101–107 (1991)

6 Kitabatake, N., Tahara, M. and Doi, E. Thermal denaturation of soybean protein at low water contents. *Agriculture Biological Chemistry*, **54**, 2205–2212 (1990)

7 Voojs, A. J., Van Der Steen, P. J. and Beumer, H. Kinetic methods for research into inactivation of antinutritional factors. In: Huisman, J., Van Der Poel, T. F. B. and Liener, I. E. (Eds), *Recent Advances of Research in Antinutrional Factors in Legume Seeds*. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation (Pudoc), pp. 272–275 (1989)

8 Wolf, W. J., Peterson, R. E. and Schaer, M. L. Preparative reversed-phase high-performance liquid chromatography and gel electrophoresis of soybean proteins. *Journal of Agricultural Food Chemistry*, in press

9 SAS. *SAS/STAT Guide for Personal Computers. Version 6 Edition*. Cary, NC: SAS Institute Inc., p. 1028 (1987)

10 Tanteeratarm, K., Wei, L. S., Steinberg, M. P. and Yamashita, N. Bound water associated with 7S and 11S soy proteins determined by vapor sorption isoterms and pulsed NMR. *Journal of Food Science*, **55**, 130–132 (1990)

11 Hoshi, Y. and Yamauchi, F. Determination of sulfhydryl and disulfide contents of soybean 11S globulin and their change by lyophilization. *Agricultural Biological Chemistry*, **47**, 2435–2440 (1983)

12 Hoshi, Y., Yamauchi, F. and Shibasaki, K. Effects of relative humidity on aggregation of soybean 7S and 11S globulins. *Agricultural Biological Chemistry*, **46**, 1513–1517 (1982)

13 Hoshi, Y. and Yamauchi, F. Further studies on aggregation and insolubilization of soybean 11S globulin with humidity during storage. *Agricultural Biological Chemistry*, **47**, 1473–1479 (1983)

14 Shimada, K. and Cheftel, J. C. Determination of sulfhydryl groups and disulfide bands in heat-induced gels of soy protein isolate. *Journal of Agricultural Food Chemistry*, **36**, 147–153 (1988)

15 MARSHALL, W. E. AND ZARINS, Z. M. Exothermic transitions of glycinin determined by differential scanning calorimetry. *Journal of Agricultural Food Chemistry*, **37**, 869–873 (1989)
16 BIKBOV, T. M., DANILENKO, A. N., PLETENKO, M. G., GERMAN, M. L., VARFOLOMEEVA, E. P., GRINBERG, V. YA., LEONYTEV, A. L. AND TOLSTOGUZOV, V. B. Selective thermal denaturation as a method of preparative isolation of 11S globulins from plant seeds. *Journal of Agricultural Food Chemistry*, **34**, 297–302 (1986)
17 FUJITA, Y. AND NODA, Y. Effect of hydration on the thermal stability of protein as measured by differential scanning calorimetry. Chymotrypsinogen A. *International Journal of Peptide and Protein Research*, **18**, 12–17 (1981)
18 RUEGG, M., MOOR, U. AND BLANC, B. Hydration and thermal denaturation of β-lactoglobulin. A calorimetric study. *Biochimica et Biophysica Acta*, **400**, 334–342 (1975)
19 HAGERDAL, B. AND MARTENS, H. Influence of water content on the stability of myoglobin to heat treatment. *Journal of Food Science*, **41**, 933–937 (1976)
20 LUESCHER, M., RUEGG, M. AND SCHINDLER, P. Effect of hydration upon the thermal stability of tropocollagen and its dependence on the presence of neutral salts. *Biopolymers*, **13**, 2489–2503 (1974)